

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Patrice Marche <i>et al.</i>)	Confirmation No. 7396
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Application No. 10/581,814)	Art Unit: 1637
)	
Filed: August 22, 2007)	Examiner: S. C. Woolwine
)	
For: Method for quantitative evaluation of a rearrangement or a targeted genetic recombination of an individual and uses thereof)	
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DECLARATION UNDER 37 C.F.R. § 1.132

I, the undersigned, Nicolas PASQUAL, do hereby declare that:

1. I am a citizen of France, residing at 43, allée des Romantiques, 38100 Grenoble, FRANCE.
2. I am currently employed at Immun*ID. My *curriculum vitae* is enclosed.
3. I am an inventor of U.S. Patent Application 10/581,814, which I fully understand. I have reviewed the Office Action, dated February 23, 2011. I have also read the cited references used to allege that the claimed invention is obvious, as set forth in the Office Action dated February 23, 2011. I believe that the feature of determining a TRAD rearrangement from a sample of human genomic DNA by multiplex PCR is not obvious over these references (please note that "TRAD" is the present notation, proposed by IMGT, for what was called "TCRAD" before).
4. The Office Action of February 23, 2011, alleges that the methods of Pasqual (Pasqual *et al.*, J. Exp. Med., 2002) can be obviously adapted to perform multiplex PCR on a sample of 50-100 ng of human genomic DNA to amplify fragments up to 24.2 kilobases as disclosed by Komuldin with elongation steps of 12 minutes, incremented by 15 seconds per cycle, and expect to directly visualize an obtained product in a gel stained with ethidium bromide. By rendering this rejection, the Examiner forgot one very important feature of our method, which is that the amplified fragments are fragments of genomic DNA (gDNA) resulting from different VJ rearrangements of the genes of the TRAD locus. This particular feature of the fragments which are amplified in the claimed method renders their amplification largely more difficult than that of a non-rearranged gene, present in two copies in each human diploidic cell.

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5. As is perfectly known by the skilled artisan, and explained in the specification of the application, the great variety of the immune repertoire is generated by several mechanisms, especially during V(D)J recombination. Briefly, the genes of the antigen receptors are discontinued in the genome and need to recombine to become active. The recombination leads to a combinatorial diversity, corresponding to the gene segments which are rearranged. Another level of diversity, called junctional diversity, results from the addition and/or deletion of base(s) during recombination. Our method measures combinatorial diversity.

6. When the invention was made, the TRAD locus was known to comprise 41 families of V gene segments, and at least 61 J genes, among which 50 were functional (see Hodges *et al.*, enclosed, page 1, third paragraph of the right column). To date, it is admitted that the TRAD locus comprises 41 functional V gene families, and 48 functional J genes (out of 61 J genes), which rearrange together. This leads to $41 \times 48 = 1968$ rearrangements.

7. In my article published before the filing of the application, and cited by the Examiner (Pasqual *et al.*, *supra*), we studied the V-J α rearrangements during mouse thymocytes differentiation, and noted that preferential associations exist between the V α and J α segments according to their position in the locus. More precisely, we observed that for a given V α segment, the J α segments are used with a Gaussian distribution centered on about 15 J α segments (see page 1170, last paragraph of the right column). This means that a given V α gene segment rearranges most often with one of 15 J α gene segments, and that other J α genes, upstream and downstream of these 15 preferred genes, are also used, although less frequently. Hence, the frequency of any given V-J rearrangement is not precisely $1/1968$, but can be around $1/(41 \times 15) = 1/615$ for the most frequent ones, and far less for the less frequent ones.

8. In the application, we also mention (page 5) two other mechanisms leading to the fact that the real diversity is inferior to the theoretical diversity of 10^{15} :

(i) the structural compatibilities between subunits: this mechanism has no impact on the combinatorial diversity studied at the genomic level; and

(ii) negative selection in the thymus: this selection aims at eliminating T clones which exhibit a too strong "self reaction". Since this specificity essentially results from the tridimensional structure of the TCR at the CDR3 level, this negative selection mostly impacts the junctional diversity mentioned above, rather than the combinatorial diversity which is studied by our method.

9. From the above, we can over-estimate the frequency of a given rearrangement as follows: $1/615$ for the most frequent ones, and $1/1968$ for the less frequent ones. This means that, to have ONE

copy of template for a given rearranged gene, between more than 615/2 and more than 1968/2 copies of diploidic genome are necessary, whereas, for a non-rearranged gene such as the β -globin gene amplified by Cheng *et al.* from a sample of 37 ng of genomic DNA, this gene is present two exemplars in each copy of the genome. Hence, for one of the most frequent rearranged genes, at least 37×615 ng = 22.755 μ g of genomic DNA are necessary to have the same amount of template as in Cheng *et al.* (in terms of copy number, which is the relevant feature for PCR amplification).

10. In my former Declaration, I have shown that the efficiency of the PCR decreases when high concentrations of DNA are used. In particular, according to my experience, but also to the general knowledge of any skilled artisan, the PCR is strongly inhibited when the total amount of DNA in the tube is 2 μ g. Hence, in view of the prior art, one skilled in the art would not be motivated to attempt to perform methods for producing a PCR fragment as long as 35 kilobases (as disclosed by Barnes), or even 22.4 kilobases (as disclosed by Cheng) by amplifying a rearranged V-J gene with human gDNA as starting template. The skilled artisan would not have expected that such an amplification was possible, and even less that the amplified product could be detected directly on a gel. Accordingly, at the time of filing of the present application, the claimed invention was not obvious.

11. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 21/06/2011
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By: Nicolas PASQUAL

